Immature stages of the Neotropical skipper *Lychnuchoides ozias ozias* (Hewitson, 1878) (Lepidoptera: Hesperiidae)

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Abstract: The early stages of the hesperiid butterfly *Lychnuchoides ozias ozias* (Hewitson) are described. The food plant in nature is an unidentified species of Marantaceae (*cf*. *Calathea* G. F. W. Meyer). The egg is hemispheric, white, with about 50 vertical ribs. The larva is smooth and goes through five instars during 30 days. The last instar has a plump body, is completely smooth and dark green with a pair of dorsal light markings on T6, the head is dark brown, trapezoidal, with a pair of rounded bumps on the vertex with two pairs of yellow spots (one frontal and one on the bumps on the vertex). The pupa is elongated, without spines, bearing a long, thin, anteriorly-directed projection on the head. The immature stages are similar to those of most Calpodini except for the general body shape, which is similar to *Talides* Hübner and *Saliana* Evans and distinct from all other Calpodini genera, whose larvae are elongated and slim. The body shape in Calpodini appears to be associated with host plant use, with plump bodies related to the use of plants with large soft leaves and slender bodies related to the use of plants with narrow and tough leaves or leaflets.

Key words: Atlantic Forest, Calpodini, Hesperiinae, Marantaceae

Resumo: Os estágios imaturos da borboleta *Lychnuchoides ozias ozias* (Hewitson) (Hesperiidae) são descritos. A planta de alimento é uma espécie não identificada de Marantaceae (*cf*. *Calathea* G. F. W. Meyer). O ovo é hemisférico, branco, com cerca de 50 estrias verticais. A larva é lisa e passa por cinco instares ao longo de 30 dias. O último instar possui corpo rechonchudo, completamente liso, é verde escuro com um par de manchas claras no segmento T6, a cabeça é marrom escura, trapezoide, com um par de protuberâncias no vertex e dois pares de manchas amarelas (um par frontal e o outro nas protuberâncias do vertex). A pupa é alongada, sem espinhos, e apresenta um prolongamento fino apontando para a frente na cabeça. Os estágios imaturos são similares aos da maioria dos Calpodini, exceto pela forma geral do corpo, que é mais similar a *Talides* Hübner and *Saliana* Evans, sendo distinta de todos os outros gêneros de Calpodini, que possuem corpo fino e alongado. A forma do corpo nos Calpodini parece estar associada com a planta hospedeira larval onde corpos rechonchudos são relacionados ao uso de plantas com folhas largas e macias enquanto corpos finos são associados com o uso de plantas com folíolos estreitos e rígidos.

Palavras chave: Calpodini, Hesperiinae, Marantaceae, Mata Atlântica

INTRODUCTION

With more than 4,000 described species, Hesperiidae is the second richest butterfly family, although it is one of the least known concerning its systematics and natural history, including knowledge of larval food plants and descriptions of the immature stages (Warren, 2000; Warren *et al.*, 2008). In the Neotropics, for example, after the impressive work of Moss (1949) reporting food plants or immature stages of almost 200 species from Pará, the immature biology of Neotropical skippers was neglected for almost four decades. Recently, however, a series of detailed descriptions of immature stages have been published adding important information for this diverse butterfly family (see Burns and Janzen, 1999, 2001; Greeney & Warren, 2003, 2004, 2009 a,b; Bächtold *et al.*, 2012, 2017; Moraes *et al.*, 2012; Lepesqueur *et al.*, 2017 and references therein).

The genus *Lychnuchoides* Godman (Hesperiidae, Hesperiinae, Calpodini) is composed of three very similar species occurring from Mexico to Argentina (Evans, 1955; Mielke, 2004, 2005; Constantino *et al.*, 2013; Warren *et al.*, 2018). With two described subspecies, *Lychnuchoides ozias* (Hewitson) occurs from Ecuador to northern Argentina (Mielke, 2005; Warren *et al.*, 2018). The nominal subspecies *Lychnuchoides ozias ozias* (Fig. 1) is present in several localities of the Atlantic Forest in southern and southeastern Brazil, and is usually associated with dense humid forests, resting and feeding in shaded habitats (Brown, 1992; pers. obs.). Despite the large amount of data that has recently become available, however, no data on food plants or immature stages are available for the genus *Lychnuchoides* (*e.g*. Janzen and Hallwachs, 2018 for data on immature stages and Beccaloni *et al.*, 2008 for larval food plants). The aim of the present paper is to describe the early stages and report a larval food plant for *Lychnuchoides ozias ozias* in southeastern Brazil.

STUDY SITE AND METHODS

A single egg was collected on November 1, 2005 at "Parque das Neblinas", a private forest reserve in Taiaçupeba District, Mogi das Cruzes, São Paulo State (23°44'S 46°10'W; 800 m). The region is covered by montane rain forest (Ururahy *et al.*, 1987) with an annual rainfall of over 3,000 mm and average annual temperature of 20 °C (Nimer 1972). The larva was reared at room temperature (around 19 °C and 60% humidity) in a 500 ml plastic container with pieces of mature leaves of an unidentified Marantaceae (*cf*. *Calathea* G. F. W. Meyer) collected in the field where the egg was oviposited, and were offered *ad libitum*. The larva was checked daily to replace the food and to clean its container. Head capsules were collected and kept for measurements. The duration of instars and pupal development were recorded. Measurements were taken and general aspects of morphology were observed using a stereomicroscope equipped with a micrometric scale. Egg "size" is recorded as height and diameter. The total length of the larva and pupa were measured in dorsal view. The larval head capsule width is the distance between the most external ocelli (as in Freitas, 2007). Color patterns of immature stages *in vivo* were photographed with a digital camera. Adults, capsules, and pupal skins are deposited in the entomological collection of the Universidade Estadual de Campinas (Museu de Zoologia "Adão José Cardoso", ZUEC).

RESULTS

Natural history. A single egg was obtained from a wild ovipositing female. Oviposition was observed on November 1, 2005, a rainy day, at 16:00, when the temperature was 14.7 °C. After touching several broad leaves while flying fast near the ground, a female spent about five minutes circling an unidentified species of Marantaceae (possibly a species of *Calathea* G. F. W. Meyer) before ovipositing a single white egg on the upper surface of a mature leaf (Fig. 1A). Due to potential artifacts resulting from rearing the larva in plastic pots with pieces of the food plant, shelter-building behavior could have been affected (see Greeney & Warren, 2009b). The first instar built a simple shelter by rolling a small portion of the leaf margin either onto the upper or lower surface of the leaf, forming a loose, tubular shelter. In later instars and for pupation (see below), shelters were built by rolling most of or the entire leaf fragment into a tube. Frass ejection was not observed, although this behavior cannot be supposed to be absent. Pupation occurred inside a non-cut shelter consisting of the rolled apical portion of a mature leaf with the aid of two strengthened strands of silk (Fig. 1K). The duration from egg hatching to adult emergence was of 43 days.

Description of immature stages

Egg (Fig. 1A,B). Hemispherical, white, with about 50 vertical ribs (transverse ribs not visible under light microscopy); height 1.0 mm, diameter 1.3 mm. Duration: 8 days.

First instar (Fig. 1C-E). Body completely smooth, tegument whitish translucent; after feeding, body becomes greenish; head capsule black, trapezoidal, with a pair of rounded bumps on vertex. Head and body tegument smooth, without scoli or projections; a black prothoracic plate dorsally on T1. Legs and prolegs light. Head capsule width: 0.8 mm, height 0.8 mm; maximum body length 7 mm. Duration: 3 days.

Second instar (Fig. 1F). Body completely smooth, tegument pale yellow translucent; head dark brown, trapezoidal, with a pair of rounded bumps on vertex. Intestinal contents dark green in color and visible. Legs and prolegs light. Head capsule width: 1.18 mm, height 1.24 mm; maximum body length 12 mm. Duration: 4 days.

Third instar (Fig. 1G). Body plump, completely smooth, tegument dark green translucent with a pair of dorsal light markings on T6, tracheal system visible forming a lateral white line following spiracles; head dark brown, trapezoidal, with a pair of rounded bumps on vertex. Legs and prolegs light. Head capsule width: 1.62 mm, height 1.80 mm; maximum body length 17 mm. Duration: 4 days.

Fourth instar (Fig. 1H). Body plump, abruptly decreasing in diameter in thoracic segments towards head, completely smooth, dark green translucent with a pair of dorsal light markings on T6, tracheal system visible forming a lateral white line following spiracles, head similar to previous instar, dark brown with two pairs of yellow spots, one frontal and one on bumps on vertex. Legs and prolegs light. Head capsule width: 2.21 mm, height 2.58 mm; maximum body length 30 mm. Duration: 7 days.

Fifth (last) instar (Figs. 1I-K). Early fifth instar (Fig. 1I) very similar to previous instar. Late fifth instar (Fig. 1J) body light variegated bluish, translucent; pair of dorsal markings on T6 became yellow, tracheal system visible forming a lateral white line following spiracles; head similar to previous instar but elongated, dark brown with two pairs of yellow spots, one frontal and one on bumps on vertex. Legs and prolegs light. Pre-pupa (Fig. 1K) had a translucent light green aspect. Head capsule width: 2.9 mm, height 3.6 mm; maximum body length 50 mm. Duration: 12 days.

Pupa (Fig. 1L,M). General shape elongated, without spines, head bearing a long, thin, anteriorly-directed projection; proboscis sheath same length as body. Attached to leaf by two silk girdles, one at cremaster and other at thoracic region. General color light green. Maximum length 37 mm. Duration: 8 days.

DISCUSSION

In the most recent taxonomic arrangement of Hesperiidae, the genus *Lychnuchoides* was placed in the tribe Calpodini based on morphological evidence since no molecular data for the genus is available (Warren *et al.*, 2009). In fact, the immature stages are similar to those of most Calpodini whose immature stages are known so far, including the shape of the head capsule in the last instars and the slender pupae with the anteriorly-directed projection on the head. However, considering the body shape of the last instars, *Lychnuchoides* caterpillars are distinct to those of most Calpodini genera. The main difference is the body shape of the last instars; larvae of *Lychnuchoides*, *Talides* Hübner and *Saliana* Evans (and to some extent *Calpodes* Hübner) present a plump body, at least twice the height of the head in late last instar, abruptly decreasing in diameter in thoracic segments towards the head. In almost all other putative Calpodini genera (such as *Argon* Evans, *Carystina* Evans, *Carystoides* Godman, *Carystus* Hübner, *Cobalus* Hübner, *Dubiella* Evans, *Ebusus* Evans, *Nyctus* Mabille, *Panoquina* Hemming, *Synale* Mabille, *Tromba* Evans, *Thracides* Hübner), the larval body shape is elongated and slim, just slightly wider than the head (Moss, 1949; Janzen & Hallwachs, 2018; unpublished data). The last instar of *Tisias* Godman also presents a plump body, but it is quite distinct from *Lychnuchoides* by being furry and by not showing the abrupt decrease in diameter in the thoracic segments (see Janzen & Hallwachs, 2018).

Intriguingly, body shape seems to be associated with host plant use; while *Lychnuchoides*, *Talides*, *Saliana* and *Calpodes* primarily feed on Musaceae, Heliconiaceae, Marantaceae and other monocots with large and soft leaves, all remaining genera (except *Thracides*) feed primarily on Arecaceae and Poaceae,

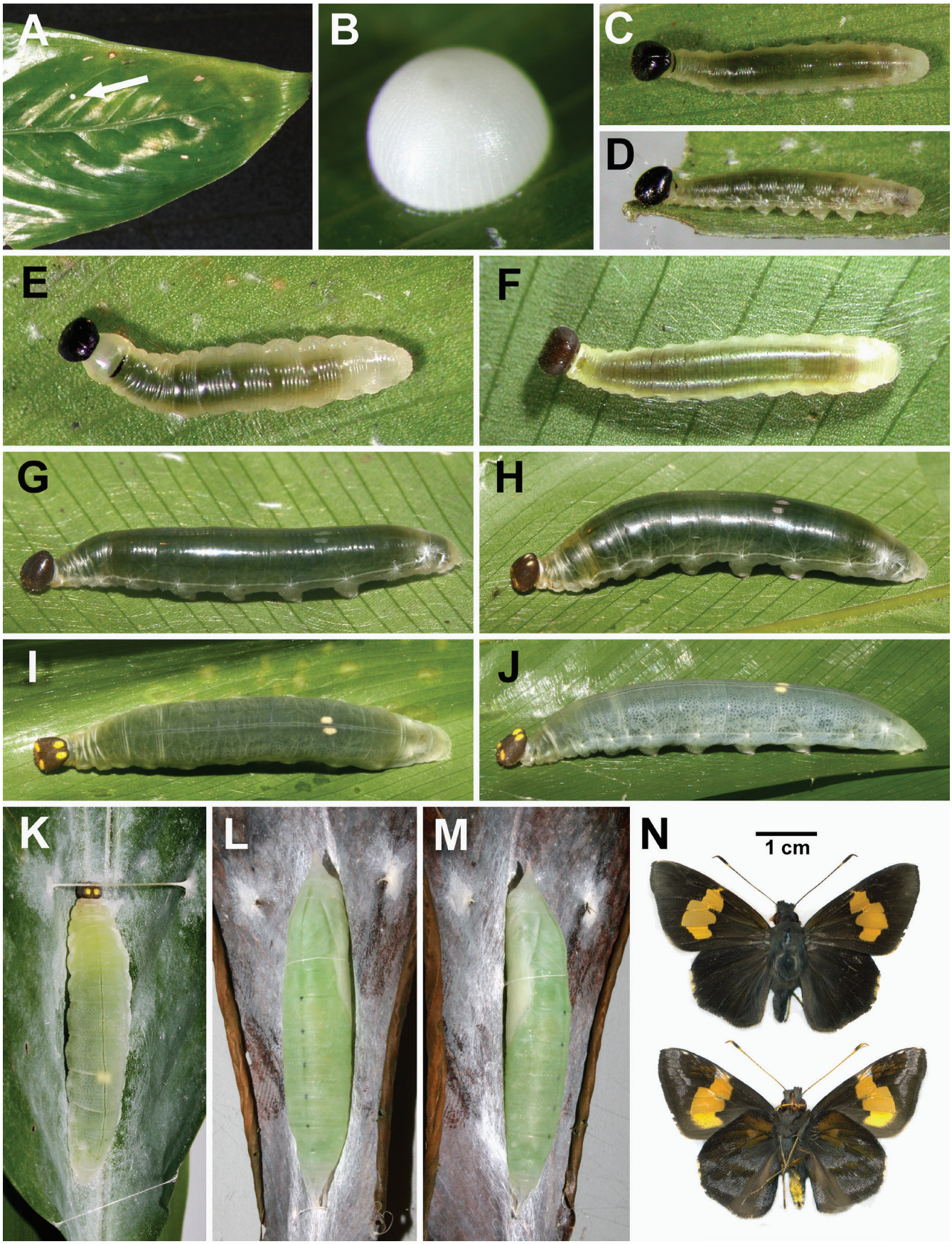

Fig. 1. Life stages of *Lychnuchoides ozias ozias* on an unidentified Marantaceae (*cf. Calathea*) in "Parque das Neblinas", Mogi das Cruzes, São Paulo, Brazil. A, egg on upper leaf (white arrow); B, close lateral view of egg; C, D, early first instar; E, late first instar, dorsal; F, second instar, dorsal; G, third instar, lateral; H, fourth instar, lateral; I, early fifth (last) instar, dorsal; J, late fifth (last) instar, lateral; K, prepupa, dorsal; L, M, pupa in dorsal and lateral views, respectively; N, adult male (dorsal above, ventral below).

most with slender and tough leaves and leaflets (Beccaloni *et al.*, 2008; Moss, 1949; Janzen & Hallwachs, 2018; unpublished data). Although the tribe Calpodini was recovered as a monophyletic group by Warren *et al.* (2009), recent studies showed that this could be polyphyletic, with up to four distinct linneages (Sahoo *et al.*, 2016; see also Espeland *et al.*, 2018). However, the fact that those genera whose larvae present plump body shape are spread in different lineages of "Calpodini" *sensu* Sahoo *et al.* (2016) reinforce the hypothesis that leaf traits could be the key factor explaining body shape of larvae of these groups. In conclusion, although not investigated in detail, leaf shape and toughness are possibly related to shelter construction in Hesperiidae. Thus, it would be interesting to investigate whether host plant use and shelter type are constrained by body shape or vice versa.

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